

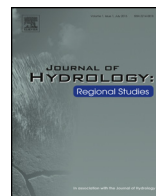


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Chemistry of natural waters and its relation to Buruli ulcer in Ghana



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ABSTRACT

Study region: Buruli ulcer, an emerging disease caused by *Mycobacterium ulcerans*, largely affects poor rural populations in tropical countries. The environmental niche that supports this necrotizing bacterium is unclear. Here, water samples were collected from five communities within Ghana in the rainy season in 2011: four in the southern part of Ghana (three disease-endemic communities: Pokukrom, Betenase, and Ayanfuri, and one control: Kedadwen) and one non-endemic community (Nangrumba) in the north.

Study focus: Past studies of Buruli ulcer conclude that water quality is, in some way, closely related to the transmission of this disease. This work serves as a first step to explore links between Buruli ulcer incidence and water quality. More broadly, this research works toward identifying the environmental niche for *M. ulcerans*, providing characterization of water bodies hazardous to human health in at-risk communities.

New hydrological insights: Trace metals, thought to aid in the preferential growth of *M. ulcerans*, are present in higher concentrations in mining pits and stagnant pools than in other tested water bodies. Arsenic in particular could serve as a double threat for BU incidence: it could support the growth of *M. ulcerans* while suppressing immune systems, making the population more susceptible to disease. Few other differences between endemic and non-endemic communities exist, implying other variables such as human behavior may also control the onset of Buruli ulcer.

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1. Introduction

The Millennium Development Goals were proposed in 2000 by the United Nations to improve quality of life in developing countries by 2015 (United Nations, 2000). Included in this list is a goal to combat major diseases such as HIV/AIDS, malaria, and tuberculosis. Lost in this list is a class of diseases known as neglected tropical diseases. Neglected tropical diseases share a common theme: while disproportionately affecting the world's poorest and most marginalized populations (as do malaria and tuberculosis), they receive much less funding for research and treatment than more well-known diseases (WHO, 2010). One such disease is Buruli ulcer (BU), a necrotizing skin disease caused by *Mycobacterium ulcerans*, which is the third most common Mycobacterial disease after tuberculosis and leprosy (Merritt et al., 2005). This disease is an enigma for scientists, as its mode of transmission is unknown. Treatment is well understood clinically, although in practice, it is expensive, long, and often poorly managed (WHO, 2012a,b; Hausermann et al., 2012). Recently, many scientists have turned their attention to determining the mode of transmission for BU in an effort to prevent this disease; this is the World Health Organization's top research priority (WHO, 2012a,b). Cases of BU have been reported in over 30 tropical and subtropical countries, typically in poor rural communities, and most frequently in West Africa (WHO, 2007). While BU is typically non-fatal, it can result in severe deformity and medical complications if not promptly and properly treated.

An extensive body of research suggests that BU is prevalent in areas subject to rapid environmental modification such as logging, irrigation, agriculture, mining, or dam construction (Hayman, 1991; Veitch et al., 1997; Merritt et al., 2005, 2010; Wagner et al., 2008). Still others have found that direct contact with aquatic environments is a major risk factor (Aiga et al., 2004; Raghunathan et al., 2005; Debacker et al., 2006; Sopoh et al., 2010), and *M. ulcerans* has been detected in fish (Eddyani et al., 2004), aquatic snails (Marsollier et al., 2004), aquatic insects (Portaels et al., 1999), and submerged terrestrial plants (McIntosh et al., 2014). While the culturing of *M. ulcerans* from an aquatic invertebrate (Portaels et al., 2008) suggested a potential reservoir or vector for BU (e.g., Merritt et al., 2010), Benbow et al. (2008) discounted predatory aquatic insects as a potential vector because the number of insects and presence of *M. ulcerans* in these insects are similar at BU-endemic and non-endemic sites. However, more recent work indicates that perhaps aquatic biting insects are indeed the cause, as suggested by correlations between biting insect distribution and prevalence of BU (Carolan et al., 2014a) and, more directly, one case study of a six-year old girl (Marion et al., 2014).

Importantly, in a recent country-wide survey in Ghana, Benbow et al. (2013) often found presence of *M. ulcerans* in water bodies in BU-endemic regions, but not in non-endemic regions. Garchitorena et al. (2014) explored the spatial and temporal variation of *M. ulcerans* in a variety of aquatic ecosystems. They found that while low oxygen, high temperature swamps were a preferred location for *M. ulcerans*, colonization dynamics in aquatic systems varied throughout the year and were correlated to rainfall. Their results indicate that the environment may be more favorable to the bacteria at certain times of year and that transmission is a complex interplay between the biota and the ecology of the system. Merritt et al. (2005) proposed that "poor water quality influences biological communities, leading to increased growth and proliferation of *M. ulcerans* in aquatic habitats." A study of mycobacteria in brook waters, conducted by Iivanainen et al. (1993), found that culturable counts of slow-growing mycobacteria were most negatively correlated to pH. Likewise, counts were most positively correlated to chemical oxygen demand and metals concentrations. One might expect *M. ulcerans*, a slow-growing mycobacterium, to thrive in similar environments to those bacteria studied by Iivanainen et al. (1993). Previous work has shown that the optimal pH growth range of *M. ulcerans* varies between 5.4 and 7.4 (Portaels and Pattyn, 1982), and that unlike many other mycobacteria, *M. ulcerans* has a preference for low oxygen conditions (Palomino et al., 1998; Garchitorena et al., 2014).

The assumption that *M. ulcerans* exists in environments with poor water quality is supported by many studies of BU incidence relative to land use and known chemical trends associated with these land uses (e.g., increased nitrogen in agricultural areas), and more recently, by work exploring the relationship between the distribution of *M. ulcerans* and the condition and use of the landscape upgradient

from the sampling site (Carolan et al., 2014b). Areas with high nitrogen and phosphorus concentrations will likely be a preferred environment for the growth of *M. ulcerans*, as environmental nutrient enrichment has been linked to the emergence of other direct-transmission and vector-borne bacterial diseases (Johnson et al., 2010). Duker et al. (2004) suggested a connection between arsenic enrichment in soil and water and incidence of BU. The positive correlation of mycobacterial population with metals concentrations suggests that BU incidence may be higher near mining sites, as heavy metals are commonly associated with tailing waste from mining activity (e.g., Walker et al., 2006). Taken together, previous studies about *M. ulcerans* suggest that some difference in environmental conditions, human interaction with the environment, or both, must exist between endemic and non-endemic communities to explain the difference in BU morbidity.

In this work, we explore the relations between water quality and the incidence of BU in Ghana, where more than 1000 cases of BU were reported in Ghana alone in 2010 (WHO, 2011).

This study seeks to answer the following questions: (1) Is there a difference in water quality between endemic and non-endemic communities? (2) Is there a difference in water quality between types of water bodies within these communities? and (3) If there are differences, do they relate to the postulated environment for *M. ulcerans* (high metal concentrations, low pH, high nutrient concentrations)? This study focuses on mining regions and particularly on “galamsey” (gather-and-sell), or artisanal small-scale, gold mining areas. These areas are characterized by significant localized disturbance, most notably pools of water associated with active or defunct ore-washing stations. Galamsey operations, which are poorly regulated and often unregistered and/or illegal, have no obligation to remediate spent mining areas, leading to long-term environmental degradation. Multivariate statistical methods are used to describe variation in water chemistry, water-body type, and surrounding land use. No pathogenic data are presented here and we did not directly sample for the presence of *M. ulcerans*. Rather, this work serves as a first step to explore links between Buruli ulcer incidence and the well-documented environmental niche of the bacteria. More broadly, this research works toward identifying water bodies hazardous to human health in at-risk communities.

2. Description of field sites

This study of water chemistry was conducted in five distinct communities in Ghana, West Africa (Pokukrom, Betenase, Kedadwen, Ayanfuri, and Nangruma; Fig. 1), each of which is associated with a larger study area for other related work (GIS, social science, land-use studies, etc.). These study areas comprise three endemic areas (Pokukrom, Betenase, and Ayanfuri) and two non-endemic areas (Kedadwen and Nangruma), and we aimed to control for geology, land use, and climate, as noted below. The region was selected based on data from Ghana's National BU Control Programme (2008) and through active case identification in June 2010. According to clinical records, the specific number of confirmed cases identified between 2007 and 2010 in each endemic community were 4, 4, and 14 for Pokukrom, Betenase, and Ayanfuri, respectively (Dr. Erasmus Klutse, personal communication).

2.1. Geology

All of the study communities are located in the Birimian series (Multilateral Investment Guarantee Agency, 2000), which is composed largely of volcanic rocks with gold-bearing quartz veins (Dzigbodi-Adjimah, 1993). These quartz veins contain carbonate minerals as well as metallic sulfides and arsenides. Gold-bearing quartz veins in the Birimian series were found to have high concentrations of arsenic, cadmium, copper, iron, lead, and zinc, while non-gold-bearing quartz veins showed low concentrations of these trace metals (Dzigbodi-Adjimah, 1993).

2.2. Climate

Ghana experiences two climatic seasons, rainy and dry. The rainy season occurs between May and October, while the dry season occurs between November and April. In the southern sites (Pokukrom, Betenase, Kedadwen, and Ayanfuri), annual rainfall averages 1600 mm; by contrast, Nangruma, in the northern region of Ghana, receives just 990 mm (Ghana Meteorological Service, 2010). Geographic

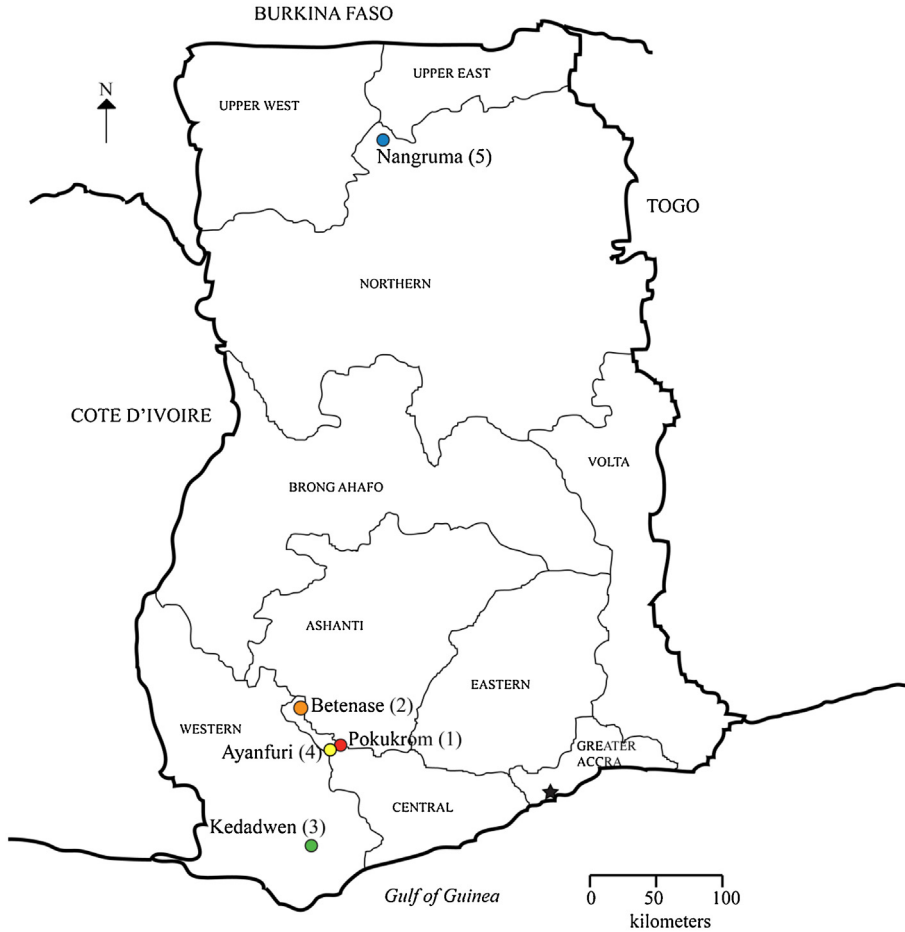


Fig. 1. Map of study areas. Buruli ulcer is endemic in Pokukrom, Betenase and Ayanfuri; Kedadwen and Nangruma are non-endemic controls.

differences also exist in the rainy season signature of these two regions: The south experiences a bimodal rainy season (major between May and July and minor between August and October), while the north sees a unimodal rainy season. Corresponding to these differences in annual rainfall, vegetation is different between these regions of Ghana: the south is characterized by thick deciduous forest, while vegetation in the north is much sparser, with shrubs and drought-resistant trees.

2.3. Agriculture

In Pokukrom, Betenase, and Ayanfuri, the major crop is cocoa, grown in plantations. As Ghana is one of the world’s leading cocoa producers, the national government subsidizes the crop and the fertilizers required to grow it effectively. Vegetables (tomatoes, eggplant, peppers, plantains, maize, etc.) are often grown in smaller polyculture plots near the cocoa crop, but some, like yams, are planted among the cocoa. In Kedadwen, rubber is the major commodity crop. Many of the same vegetables are farmed in Kedadwen as in the other southern communities, but cassava replaces yam as the major starch crop. Cassava is typically grown in monoculture fields. Nangruma, with its drier climate, has substantially different agriculture: there, yams, grown in monoculture fields in raised mounds, are the staple crop. Cattle herding is also common in Nangruma.

2.4. Mining

Gold mining is prevalent in all of these study communities, but the methods are different between northern and southern Ghana. The alluvial mining process causes more significant soil disturbance than seen in hard-rock mining. Alluvial mining is often associated with the blocking and/or rerouting of rivers to provide a water source for sediment washing or to expose riverbed sediments. Left in the wake of illegal alluvial mining is a “moonscape” of water-filled pits surrounded by mounds of mine tailings (Fig. 2a). In Pokukrom, Betenase, and Ayanfuri, mining is almost exclusively alluvial. Areas near rivers are cleared, and miners dig by hand or with excavators to expose buried river sediment deposits. These riverbed sediments are then washed and panned to extract the gold; sediments are sometimes crushed, by hand or mechanically, prior to washing and panning. Men, women, and children alike are involved in the washing and panning processes. Miners in Kedadwen also operate by surface mining, though the method is different from that of the other southern communities. The side of a hill has been excavated by multiple groups. These groups, operating individually, mine a single large tract of land; they dig and crush buried river sediments and weathered rock. Washing and panning practices are consistent with the other southern communities. By contrast, gold mining in Nangrumba is performed by hard-rock mining methods. Small shafts are dug as deep as 35 m underground. Gold-bearing ore is mined by hand or using dynamite. Ore is carried to the surface and crushed at one of several mechanical crushers in the community. Because of the occupational hazards associated with this work, miners in Nangrumba are typically adult males. The community’s river is pumped, in some cases in its entirety, to run the wet crushers and wash the crushed ore. Spent water then flows back to the river with an increased sediment load, and mine tailings are piled next to the crushers. While the landscape in Nangrumba is dotted with mine shafts, the disturbance of soils is much less than in the south.

3. Field methods

In each study community, water samples were collected from wells and boreholes, rivers and streams, galamsey mining pits, swamps, and potential “Buruli ulcer hot spots,” where applicable, in the selected community. As justified by Hausermann et al. (2012), we used an interdisciplinary perspective to include community perspectives of disease risk in selecting the perceived hot spots. “Buruli ulcer hot spots” were identified by community members during participatory mapping exercises conducted in June 2010 (Tschakert, Penn State, unpublished data). Community members indicated areas that they felt posed a risk for contracting BU; these areas were consistently pools of stagnant water. Samples of rivers and streams were taken at popular crossing points, as these are contact points common to many community members. The samples presented here were collected in June and July of 2011.

Each water sample was collected in three bottles: one 500 mL unpreserved sample for analysis of pH, major cations, major anions, and sulfate, one 500 mL preserved with H_2SO_4 for analysis of ammonia, nitrate, nitrite, and phosphate, and one 100 mL preserved with HNO_3 for analysis of trace metals. Wells and boreholes were in active production when sampled. Sample bottles were filled directly from the water body when possible; inaccessible or hazardous sites were sampled using a bucket. Highly concentrated H_2SO_4 and HNO_3 were added, as necessary, to samples immediately upon collection to achieve pH 2. All samples were stored in insulated containers until they could be transported to the laboratory at Kwame Nkrumah University of Science and Technology (KNUST). Samples were refrigerated upon arrival at the laboratory. Analysis for major ions, nitrogen, and phosphorus occurred at KNUST, while trace metal samples were sent to SGS Laboratory Services, Ghana Ltd., for analysis by ICP-OES for cadmium, copper, iron, lead, and zinc and by gaseous hydride atomic absorption (APHA 3114B) for arsenic and selenium.

3.1. Quality control/quality assurance

Error in these data are likely higher than standard sampling due to several issues in sample collection and analysis, largely unavoidable given the difficulty of sampling in this rural field area without



Fig. 2. Examples of water bodies sampled in this study. (a) Abandoned galamsey (artisanal gold-mining) pit, Pokukrom, Central region, Ghana, (b) Borehole, Nangruma, Northern region, Ghana, and (c) Hand-dug well, Pokukrom, Central region, Ghana.

significant infrastructure. Best practices for sampling, based on EPA guidelines, suggest that water samples for major ions and sulfate should be kept at 4 °C and analyzed within 28 days of collection. Similarly, samples for ammonia, nitrate, nitrite, and phosphorus should be acidified with highly concentrated sulfuric acid, kept at 4 °C, and analyzed within 28 days of collection. Samples for trace metals are less restrictive, requiring acidification with nitric acid, but allowing six months for analysis. While in the field, samples were kept in insulated containers but were not stored at 4 °C due to lack of consistent access to ice or refrigeration. Because of the remote locations of the study communities, samples were held for up to two weeks before arrival at the laboratory in Kumasi. Such difficulties are common for sampling campaigns in developing countries (Silliman et al., 2007). Upon arrival at the lab, samples were refrigerated and held for as long as 25 days before analysis for alkalinity, total hardness, sulfate, sulfide, ammonium, nitrate, nitrite, phosphate, fluoride, and manganese. Samples for trace metals were analyzed five to six months after collection.

Two types of quality control standards were employed during sampling in an effort to quantify error. Due to a lack of reliable deionized (DI) water in the field, sample bottles were filled with DI water at a laboratory in the United States and were transported to Ghana alongside clean, empty sample bottles. These trip blanks represent a proxy for field blanks. Additionally, one field duplicate was taken at each study site.

4. Statistical methods

A series of univariate and multivariate analyses were used for analysis, including one-way analysis of variance (ANOVA) and principal components analysis (PCA). ANOVA gives the measure of the contribution of an independent factor to the variance of a single variable. In this study, ANOVA was used to detect significant differences among water bodies or between endemic and non-endemic communities with respect to pH and trace metal and nutrient concentrations, the postulated target chemicals for *M. ulcerans* growth. The contribution to the variance is calculated by comparing the variance within a group to the variance between groups. Significance between groups in each ANOVA analysis was determined by Tukey's Honestly Significant Difference test. *F*-values and *p*-values are provided in the results below; *F*-values are the ratio of between-group to within-group variability and are used to assess whether the expected values of variable within community or water body differ from each other. *p*-Values indicate the significance of the results. For example, *p* = 0.05 means there is a 5% chance that the results are a false positive.

PCA reduces the number of variables in a data set to a much smaller number of synthetic variables (principal components) that represent most of the variance among samples, and was used to determine the variables that most influence the chemical signature of water bodies and to detect differences among water bodies or between endemic and non-endemic communities based on these chemical signatures. PCA calculates lines of best fit through multidimensional space, where each dimension represents a different variable. Lines of best fit are sequentially drawn perpendicular to each other until variance is completely explained. The number of best-fit lines drawn is typically less than the number of variables in the data set. Geochemical data lend themselves nicely to PCA due to linear relationships among variables and the low number of zeros in comparison to ecological data.

5. Results

5.1. pH

All water bodies tend to be slightly acidic (pH < 7) (Fig. 3a). Of the surface water bodies, BU hot spots and swamps are most consistently acidic, but wells have the most acidic waters ($F=9.2$, $p < 0.001$). This strong acidic signal in shallow groundwater is common to groundwater in Ghana and is likely related to the high acidity of soils in the region. Endemic communities (Pokukrom, Betenase, and Ayanfuri) are consistently acidic, but Kedadwen, a non-endemic community, displays similar pH values to these endemic communities (Fig. 3b). Water samples from Nangrumba are typically neutral or slightly basic, but the number of samples from Nangrumba for which pH data exist is very small ($n=3$).

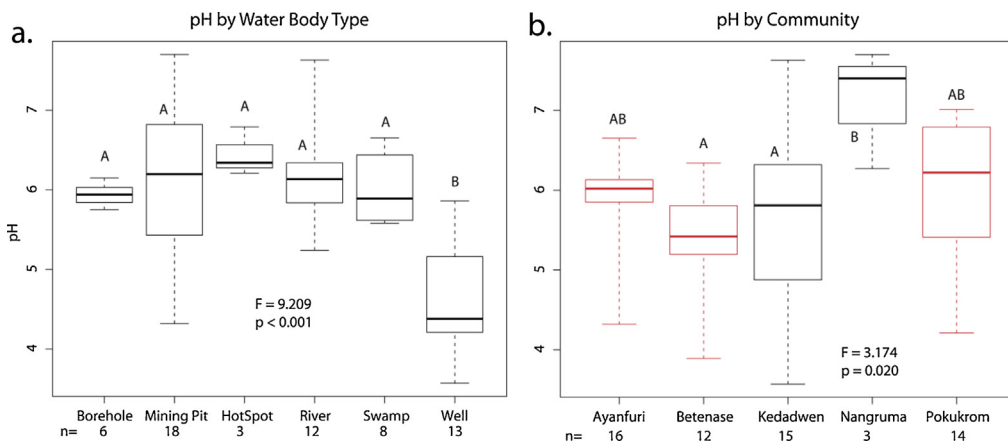


Fig. 3. Box-and-whisker plots of pH organized by (a) water body type, and (b) community. Red indicates an endemic community. n, number of samples in each group. p, statistical significance of these factors from one-way ANOVA. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of the article.)

5.2. Trace metals

We analyze for arsenic, cadmium, copper, lead, selenium, and zinc—common trace metals in these regions of Ghana. As arsenopyrite is often associated with gold deposits, it is not surprising that high arsenic concentrations are seen in galamsey pits (Fig. 4a). Arsenic concentrations are also high in BU hotspots and rivers relative to other water sources, although the differences between water bodies are not statistically significant ($F = 1.6$, $p = 0.1779$). Endemic communities (Pokukrom, Betenase, and Ayanfuri) show high arsenic concentrations, but the highest arsenic concentrations are seen in non-endemic Nangrumba (Fig. 4b). Within Nangrumba, arsenic concentrations are highest in galamsey pits.

Considering the other trace metals sorted by water body type, cadmium concentrations are highest in galamsey pits and BU hot spots ($F = 4.8$, $p < 0.001$, Fig. 4c). This trend is also seen in copper (Fig. 4e), iron (Fig. 4g), lead (Fig. 4i), and zinc (Fig. 4m). Selenium concentrations are also highest in galamsey pits and BU hot spots, but there are also elevated concentrations in some swamps (Fig. 4k). All trace metals indicate a distinct difference between galamsey mining pits and other water bodies, with no significant differences among other water bodies. Although differences among individual communities were observed in the cases of some other metals, differences were not significant between endemic and non-endemic communities (Fig. 4).

5.3. Nutrients

Nitrate concentrations are highest in boreholes, swamps, and wells (Fig. 5a). Elevated nitrate concentrations in boreholes are unexpected, as nitrate is typically sourced from the ground surface. While the boreholes in this study are presumed to be drilled into deep groundwater and are installed with a pump and intact cement pad (Fig. 1b), the wells are hand-dug and range in depth between 2 and 7 m below ground surface (Fig. 1c). These wells are typically uncased, though some wells have cement pads. A shallow water table could contribute to high nitrate concentrations as nitrate migrates into and through the subsurface from nearby agricultural fields (Chen et al., 2005). There is no significant difference between endemic and non-endemic communities (Fig. 5b). Nitrate in samples from Nangrumba is unexpected, as sampling in this community occurred prior to the growing season and thus prior to fertilizer application. Additionally, density of agricultural lands is much greater in the south; fertilizer use and corresponding nitrogen concentrations should also be greater in the southern communities.

Boreholes, representing deep groundwater, have the lowest phosphate concentrations, as phosphate is predominantly sourced from fertilizers (Fig. 5c). Ranges of phosphate concentration are

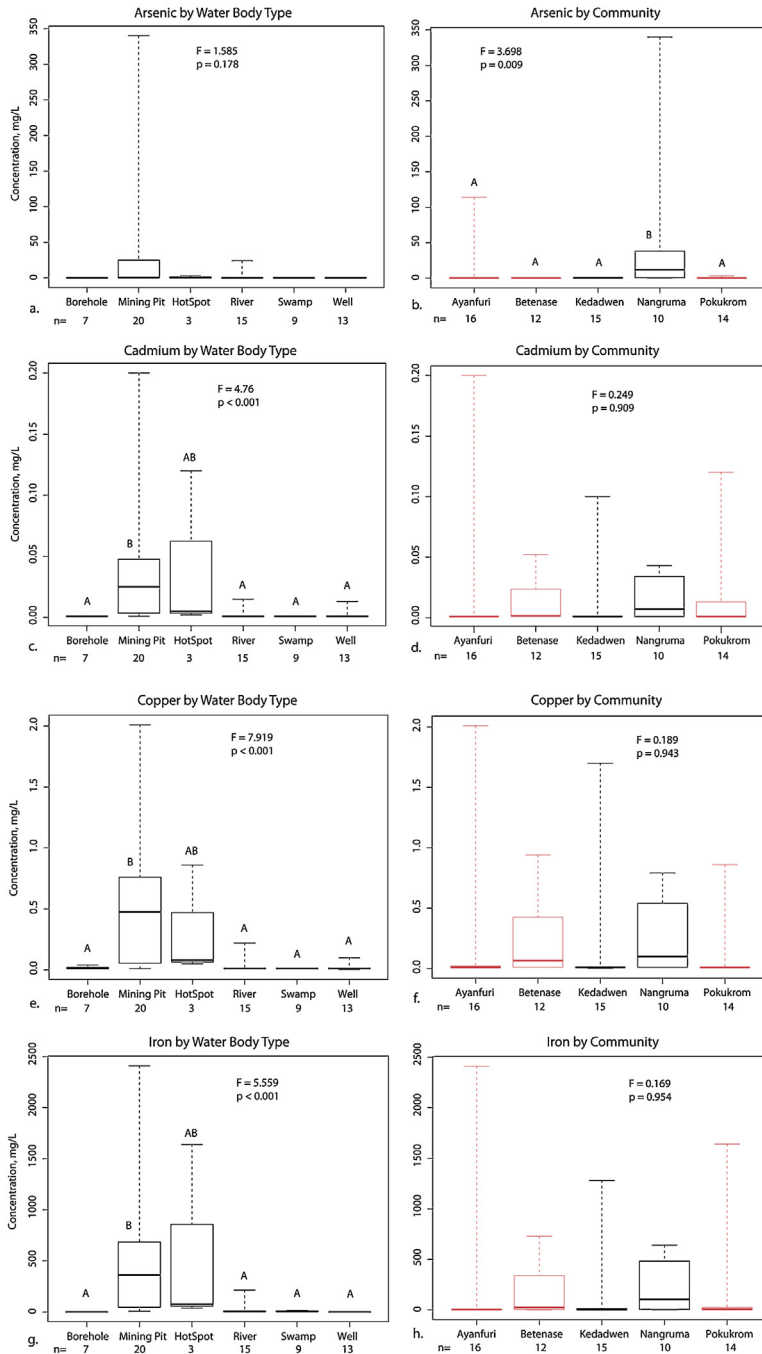


Fig. 4. Box-and-whisker plots of (a) arsenic organized by water body type and (b) by community; (c) cadmium organized by water body type and (d) by community; (e) copper organized by water body type and (f) by community; (g) iron organized by water body type and (h) by community; (i) lead organized by water body type and (j) by community; (k) selenium organized by water body type and (l) by community; (m) zinc organized by water body type and (n) by community. Red indicates endemic communities. *n*, number of samples in each group. *p*, statistical significance of these factors from one-way ANOVA. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of the article.)

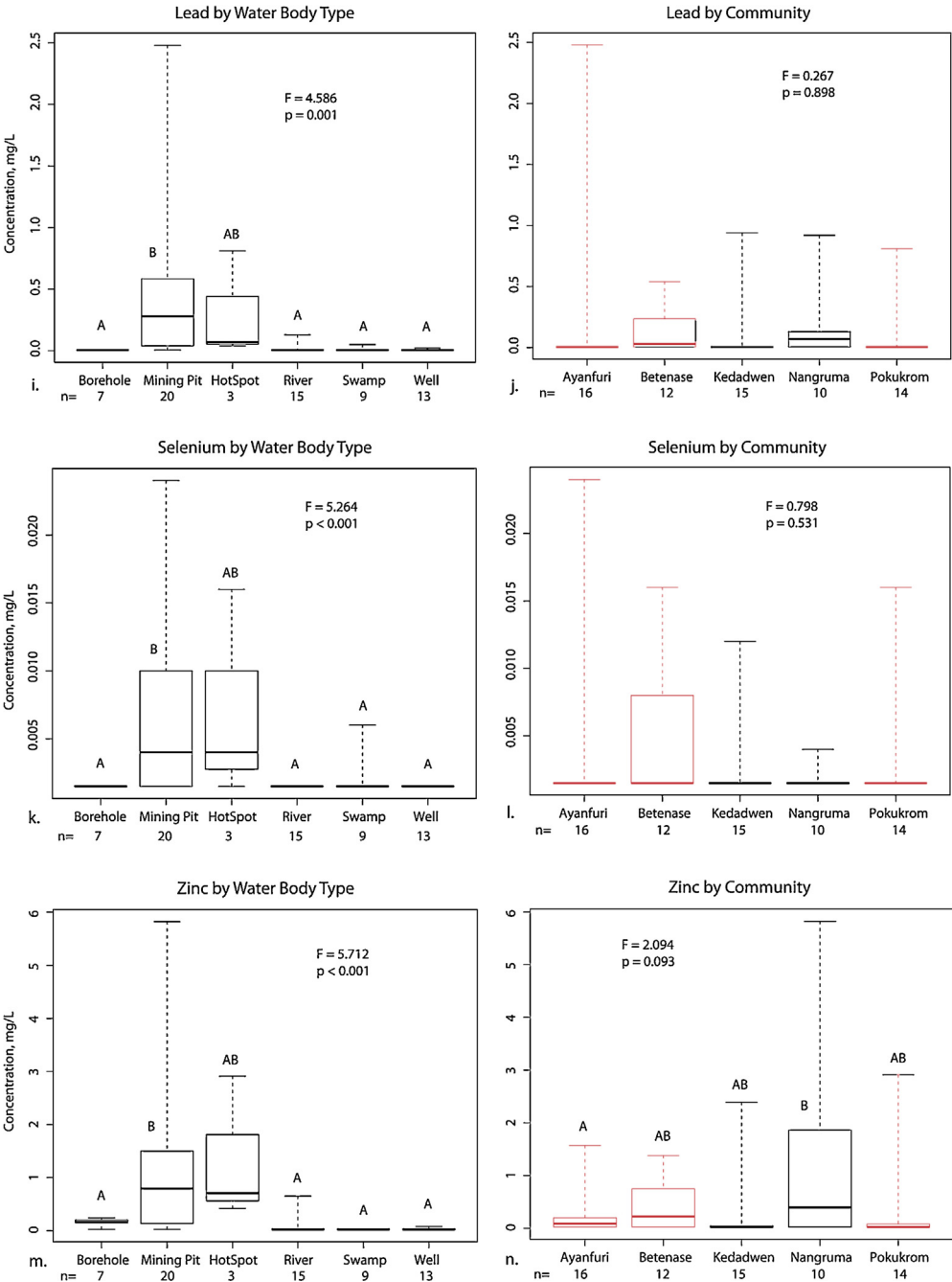


Fig. 4. (Continued).

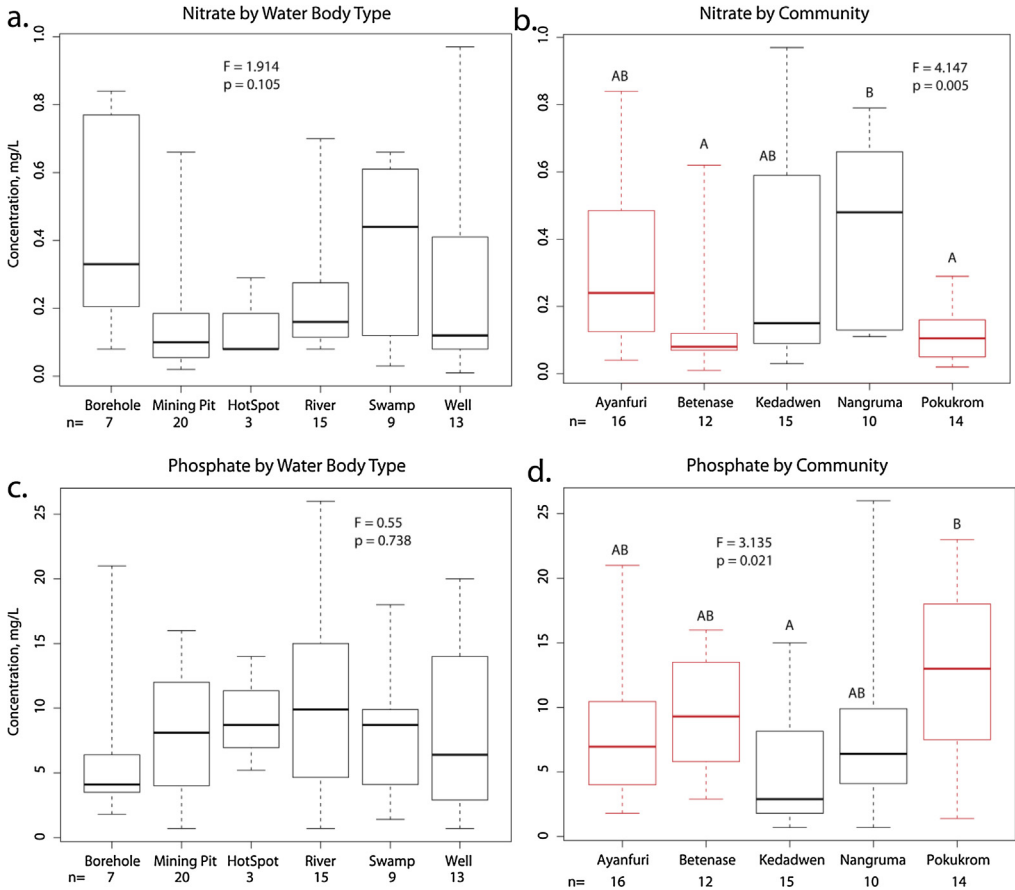


Fig. 5. Box-and-whisker plots of (a) nitrate organized by water body type and (b) by community; (c) phosphate organized by water body type and (d) by community. Red indicates endemic communities. *n*, number of samples in each group. *p*, statistical significance of these factors from one-way ANOVA. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of the article.)

similar between endemic and non-endemic communities (Fig. 5d). However, median concentrations are higher in endemic communities than in non-endemic communities.

5.4. Composite results

PCA of these data shows 41% of the variance in water chemistry is explained by principal component 1, which is dominated by trace metals (cadmium, copper, iron, lead, zinc, and selenium), a second principal component that is primarily controlled by phosphate, ammonium, and fluoride, and a third component controlled by chloride, total hardness, nitrite, and nitrate. Principal component 1 explains 28% of the total variance in the data; principal components 2 and 3 each explain an additional 13%. Full matrices of PCA scores and loadings are available through Hagarty (2012).

A few important results can be seen in the PCA analysis: (1) gamsey pits plot distinctly from other water bodies, with the division driven by their higher concentrations of trace metals relative to rivers, swamps, and groundwater (Fig. 6a), and (2) there is little difference in chemical signature between study communities (Fig. 6b). The one exception to this statement is Nangruma, which is a distinctly different climate regime than the other four sites. Interestingly, BU hot spots, those areas identified by

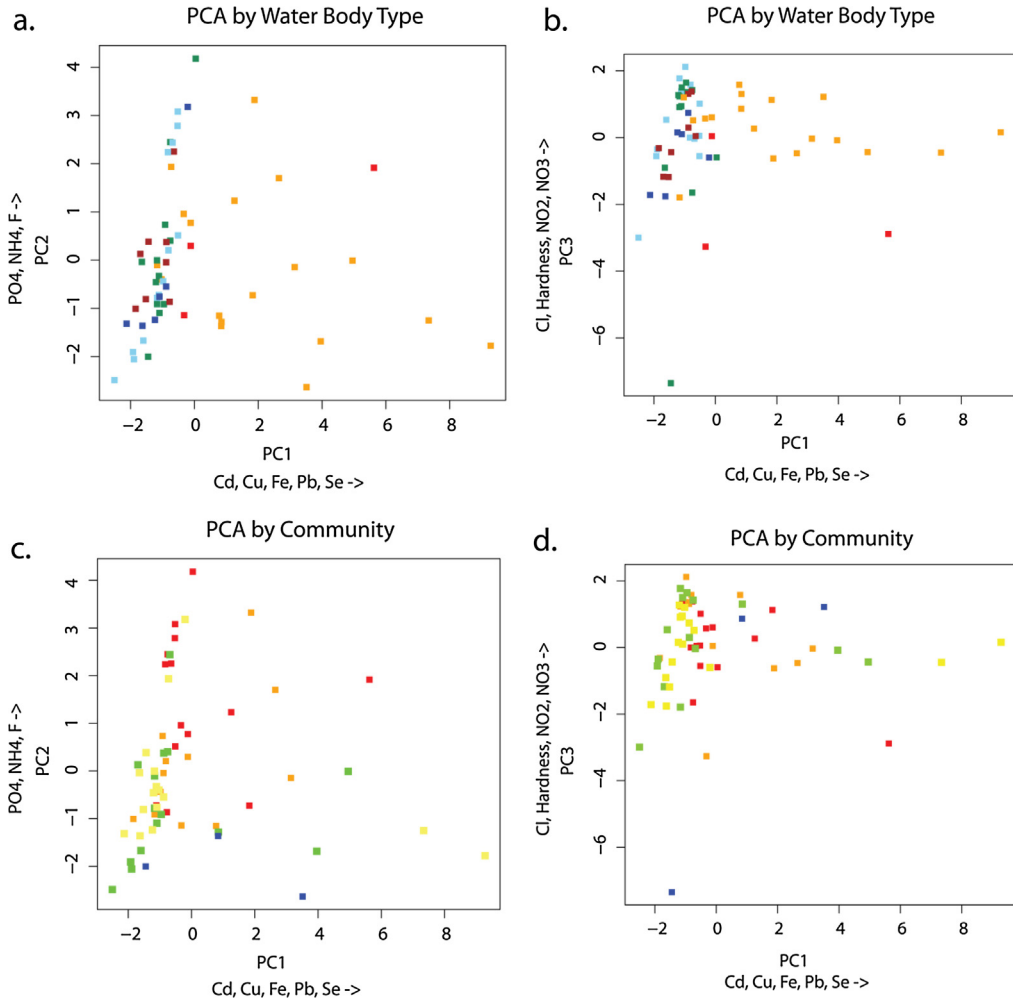


Fig. 6. PCA of June–July 2011 data, with (a) principal components 1 and 2, coded by type of water body and (b) by community; (c) principal components 1 and 3, coded by type of water body and (d) by community. Principal component 2 is dominated by PO₄, NH₄, and F; principal component 3 is dominated by Cl, hardness, NO₂, and NO₃.

community members to be “risky,” plotted more similarly with galamsey pits than with other water bodies.

Lastly, while the first principal component drives the differences between water bodies, principal component 3 seems to have no effect on these differences (Fig. 6c). As such, nitrite, nitrate, chloride, and hardness are not major factors in water body chemistry differences in that these constituents are not significantly correlated to differences in water body type. As shown in Fig. 6d, even less variation is detected among communities.

We note that laboratory quality control (method blanks and laboratory duplicates) confirm accuracy and precision of trace metal analyses, with errors typically less than three percent. The accuracy and precision of all other analyses, however, is difficult to quantify. Samples were analyzed by titration, with results representing the average of two tests for each sample. The comparison of results between field duplicate samples is variable; some duplicates display identical concentrations, while others vary by an order of magnitude. Trip blank results were very precise with respect to trace metals, but

all other analyses reveal measurable concentrations for all constituents, a matter of concern in these analyses. Fortunately, the variation among samples is much larger than the sampling and analytical error, and so the error does not significantly affect the reliability of statistical methods outlined above (e.g., Faber et al., 1993).

6. Discussion and conclusions

M. ulcerans is assumed to thrive in environments with low pH and high trace metal concentrations (e.g., Portaels and Pattyn, 1982). The analyses presented here, however, find no significant differences in pH between water bodies or among communities. The climate, land cover, agriculture, and mining practices vary somewhat among reference and endemic locations. Given the small number of sites, this variation could be important for statistical power issues that might influence negative findings. Lower pH values in southern Ghana suggest that if pH does affect the viability of *M. ulcerans*, then southern Ghana may be a more favorable growing environment than northern Ghana.

Trace metals have consistently higher concentrations in galamsey pits than in any other water body. Concentrations of cadmium, copper, iron, lead, and zinc are highest in galamsey pits and BU hot spots, and somewhat elevated in Betenase and Nangruma over other communities, which may be a function of local geology. Moreover, the finding of within-community differences in heavy metal concentrations may indicate finer-scale variation in environmental conditions that promote transmission of BU to the broader community. Ultimately, no significant difference can be seen between endemic and non-endemic communities for any trace metal with the exception of arsenic; however, arsenic concentrations in the southern communities show no statistical significance between endemic and non-endemic communities. Differences in arsenic between communities are likely caused by the differences in mining practices between these communities. In the southern communities (Pokukrom, Betenase, Kedadwen, and Ayanfuri), gold mining is largely alluvial: miners dig up and sift soil and weathered rock from riverbeds. Conversely, in Nangruma, the main mining practices are in hard rock: miners use dynamite to blast gold-bearing rock as much as 35 m underground. When this rock is brought to the surface and crushed, arsenic may be released from veins of arsenopyrite within the ore in larger quantities than it would be washed from river sediments. This arsenic may wash from galamsey drainage pits to rivers and swamps, causing a general trend of elevated arsenic concentrations in Nangruma. The connections of BU to some water quality variables may be related through human health. For example, arsenic in drinking water is known to have immunosuppressive properties (Banerjee et al., 2009); consequently, arsenic could serve as a double threat for BU incidence: arsenic in water from galamsey pits and BU hot spots could support the growth of *M. ulcerans*, while arsenic in drinking water could suppress immune systems, making the population more susceptible to BU. While this and other studies have found an association with Buruli ulcer incidence and arsenic, no laboratory or field studies have been conducted analyzing *M. ulcerans* in the presence of arsenic; other studies need to be conducted addressing *M. ulcerans* presence and abundance among these variables.

While nutrient enrichment has been linked to other bacterial diseases, there is no significant difference in nitrate concentrations between endemic and non-endemic communities in this study. However, median phosphate concentrations are higher in endemic communities than in non-endemic communities; however, it is unclear from this correlation alone whether phosphate is a variable that contributes to the growth of *M. ulcerans* in the environment.

PCA shows that differences in water chemistry are controlled by trace metal concentrations, which are somewhat correlated to the type of water body. Galamsey pits and pools of persistent stagnant water (BU hot spots) are characterized by high trace metal concentrations relative to other water bodies, though there is no significant distinction between those other water bodies (wells, swamps, boreholes, rivers). While they do not differ significantly in their nitrate and phosphate concentrations or in pH—in accordance with the assumed preferable environment for *M. ulcerans*—the high trace metal concentrations in these water bodies may harbor and promote the growth of *M. ulcerans* in the environment. No significant differences in the chemical constituents are seen between the southern communities in PCA, while the northern site of Nangruma is distinctly different from the other communities. This is likely due to the large distance (and associated climatic and geological differences) between Nangruma and the other study communities.

The results of this study are a step toward quantifying the connection between water quality and the incidence of BU; however, we note a few important limitations to this work, which are a function of the difficulty of working in this environment: (1) sampling is limited in space and time, (2) quality control of the water quality is not as good as other field systems, and (3) we have not measured the presence of *M. ulcerans* directly. Sampling directly for *M. ulcerans* is hindered by the difficulty of isolating the bacteria from environmental samples and recovering them by culture (Pettit et al., 1996; Josse et al., 1995; Portaels et al., 1997), due in large part to its sensitivity to freezing, meaning that prompt transport to the laboratory is fundamental (Meyers et al., 1974; Portaels et al., 1988). *M. ulcerans* can be detected via molecular biological techniques such as PCR (Ross et al., 1997; Fyfe et al., 2007; Portaels et al., 2008), but appears to be dispersed widely in the environment, precluding fine-scale attribution to specific BU cases or environments. Because this type of sampling is difficult in remote regions, and given these additional considerations, *M. ulcerans* was not explicitly measured in this study and the incidence of BU is instead used. Williamson et al. (2012) found that the number of *M. ulcerans*-positive samples correlated with prevalence of BU on a community scale, and we leverage that conclusion here to explore relationships between water quality and BU. Although samples were collected in only five communities, we sampled between 10 and 16 primary locations within each community, leading to significant interrogation of spatial variability at the within-community scale. However, weekly or monthly measurements of water chemistry would provide invaluable information about the temporal variability of water bodies in these study communities; however, frequent sampling at these sites is difficult due to remoteness of the study communities. Measurements of water chemistry in the wake of extreme rainfall events could also be useful; extreme rainfall and associated flooding may increase the number and size of water bodies that could harbor *M. ulcerans*. Moreover, geospatial approaches for understanding multi-scalar variability in environmental niches of the bacteria may be critical for understanding the emergence of the disease (Richardson et al., 2013; Hausermann et al., 2012). However, by identifying local-scale variability in water chemistry in BU areas, we see this work is an important step toward identifying the environmental niche of *M. ulcerans*.

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